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An Experimental Model of Calcareous Subdeltoid Bursitis Induced by Calciphylaxis

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A calcareous subdeltoid bursitis can be regularly produced in the rat through calciphylactic challenge by Thorotrast after sensitization with dihydrotachysterol (DHT). This form of calciphylaxis is described merely as an experimental model of the disease without implying that a calciphylactic mechanism is necessarily responsible for structurally comparable lesions in man.

Un subdeltoidic bursitis calcari pote esser producite regularmente in rattos per medio de un provocation calciphylactic per Thorotrast post sensibilisation con dihydrotachysterol (DHT). Iste forma de calciphylaxis es describite mermente como modello experimental del morbo, sin ulle intention de suggerer que un mechanismo calciphylactic es necessarimente responsabile pro structuralmente comparabile lesiones in le homine.

THE CALCIFYING bursitis of the shoulder joint (often referred to as subacromial or subdeltoid bursitis, bursitis chronica calcarea, periarthritis humero-scapularis or *maladie de Duplay*) is not only of great practical importance but also poses many intriguing theoretical problems as regards the biochemical and mechanical factors that may be responsible for its development. Ever since its first description by Emanuel Simon Duplay in 1896, the etiology and pathogenesis of this common and often disabling condition has been the subject of intense investigation. Its clinical course and structural pathogenesis have been largely clarified, yet virtually nothing has been learned about its etiology. Various authors considered trauma, "wear and tear," overexertion, lack of activity, infection, allergy, "rheumatism," derangements in calcium metabolism, collagen disease, or other factors to be primarily responsible for this lesion,[1-5,13] but "in spite of many explanations as a working basis, the exact method by which calcium is deposited remains obscure."[13]

An analysis of subdeltoid bursitis with exact quantitative methods has been greatly handicapped by the impossibility of reproducing in experimental

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animals any lesion even remotely resembling the clinical derangement. Experimental models of disease can offer considerable help in the elucidation of pathogenetic problems and in the search for therapeutic measures that might be applicable to the corresponding spontaneous maladies of man. Of course, a reproduction or dummy is never equivalent to the original: the metabolic derangement induced by pancreatectomy in the dog is far from duplicating clinical diabetes mellitus and the transplantable tumors of mice are not identical with the spontaneous neoplasms of man. Yet, these and many other "working models" of morbid processes proved to be useful tools in the study of spontaneous diseases. These were the considerations that encouraged us in the past to perfect various experimental similes of the so-called mesenchymal or collagen diseases in the most commonly used experimental animal, the rat.[6]

Recently we succeeded in producing an experimental model of dermatomyositis by calciphylaxis.[9] We noted that here, in addition to the inflammatory and often calcifying lesions in the skin and muscles, calcific deposits occasionally develop in the vicinity of the joints and bursae.

Calciphylaxis is a condition of induced systemic hypersensitivity in which, during a "critical period" after sensitization by a systemic calcifying factor (e.g., vitamin-D compounds, parathyroid hormone, sodium sulfathiazole), treatment with certain challengers (e.g., metallic salts, albumen) causes an acute local calcification, followed by inflammation and sclerosis. The term was coined in analogy with such designations as "anaphylaxis," "tachyphylaxis," or "skeptophylaxis," that likewise refer to induced systemic alterations in the body's responsiveness to certain challenging agents. Apparently, calciphylaxis is a fundamentally adaptive (phylactic) response that leads to defensive inflammation and sclerosis through the selective deposition of calcium in the challenged area. However, like many other basically defensive reactions (e.g., serologic immunity), it can also become the cause of morbid lesions.

Topical calciphylaxis, induced by subcutaneous injection of challengers, results in a cutaneous calcinosis reminiscent of calcareous scleroderma. However, in suitably sensitized (e.g., dihydrotachysterol- or DHT-treated) rats, calciphylactic reactions can also be elicited rather selectively, in predetermined sites (e.g., in the pancreas, bile ducts, uterus, spleen, Kupffer cells, lungs, lacrymal glands, salivary glands, or the carotid body), by the intravenous administration of challengers that have a particular affinity for one or the other organ.[8,11,12]

In our experimental model of dermatomyositis, we noted an explosive discharge and subsequent calcification of mastocyte granules. In re-examining other forms of calciphylaxis, we then observed that the scleroderma-like cutaneous and esophageal lesions produced by Thorotrast in the DHT-sensitized rat are characterized by similar mastocyte changes.[10] We therefore repeated all these experiments with various modifications in order to determine whether predominantly periarticular lesions could be obtained under certain circumstances.

It is the object of this communication to describe the conditions under which

calciphylactic sensitization with DHT, followed by challenge with Thorotrast, regularly produces a calcareous subdeltoid bursitis in the rat. We shall also see that these lesions are often accompanied by calcifying periarthritis round other joints.

Materials and Technics

Forty-five female Holtzman rats with a mean initial body weight of 210 Gm. (range 190 to 248 Gm.) were subdivided into two groups. Group 1, consisting of fifteen rats, was sensitized on the first day of the experiment by 2 mg. of DHT (dihydrotachysterol, "Calcamin"* administered in 0.5 ml. of corn oil through a rubber catheter. After 24 hours they received 1 ml. of Thorotrast, a 24 to 26 per cent stabilized colloidal thorium dioxide preparation containing 25 per cent aqueous dextrin as a stabilizer and 0.15 per cent Methyl Parasept as a preservative. This preparation is dispensed as a contrast medium for roentgenography† but was repeatedly shown to be a particularly potent calciphylactic challenger. The second group of thirty rats was similarly sensitized with DHT, but 25 hours later received 1.25 ml. of the Thorotrast solution.

During the experiment, all animals were kept exclusively on "Purina Fox-Chow"‡ and tap water. The experiment was terminated on the tenth day by sacrificing all surviving animals with chloroform. The presence of calcified periarticular lesions was verified at autopsy by naked eye inspection. Specimens of the shoulder joint, and in some instances also of other articulations, were fixed and simultaneously decalcified in Susa solution, saturated with picric acid. They were subsequently embedded in paraffin and stained with the PAS and haematoxylin-phloxine methods. Specimens of the bursae and adjacent soft tissues from the contralateral joints were carefully dissected from the bones (to obviate the need for decalcification) and fixed in alcohol-formol (8 parts of 80 per cent absolute alcohol and 2 parts of 10 per cent formaldehyde). These tissues were used for the histochemical demonstration of calcium on paraffin-embedded sections stained with the v. Kóssa technique.

Results

All animals tolerated the immediate effects of intravenous Thorotrast very well; however, 3 to 4 days after the injection they became manifestly ill and developed the previously described[10] external manifestations of calciphylaxis induced by this particular technique, that is, calcareous scleroderma-like skin changes, especially around the face, and difficulties of deglutition owing to similar lesions in the esophagus. Presumably as a consequence of these changes, eight rats of Group 1 and seventeen of Group 2 succumbed before termination of the experiment because they could not swallow an adequate amount of food. This interpretation gains support from more recent observations on similarly treated rats that were fed rice boiled in milk, instead of the usual hard Purina cubes. This soft food was easily taken and almost invariably prevented mortality, despite pronounced calciphylactic lesions.

The most striking new fact that emerged from the present experimental series was the constant development of intense periarticular calcifying lesions. The macroscopic and microscopic appearance of these morbid changes is better illustrated by photographs (figs. 1 and 2) than by words. At autopsy,

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†Testagar and Co., Detroit, Mich.

‡Purina Company of Canada.

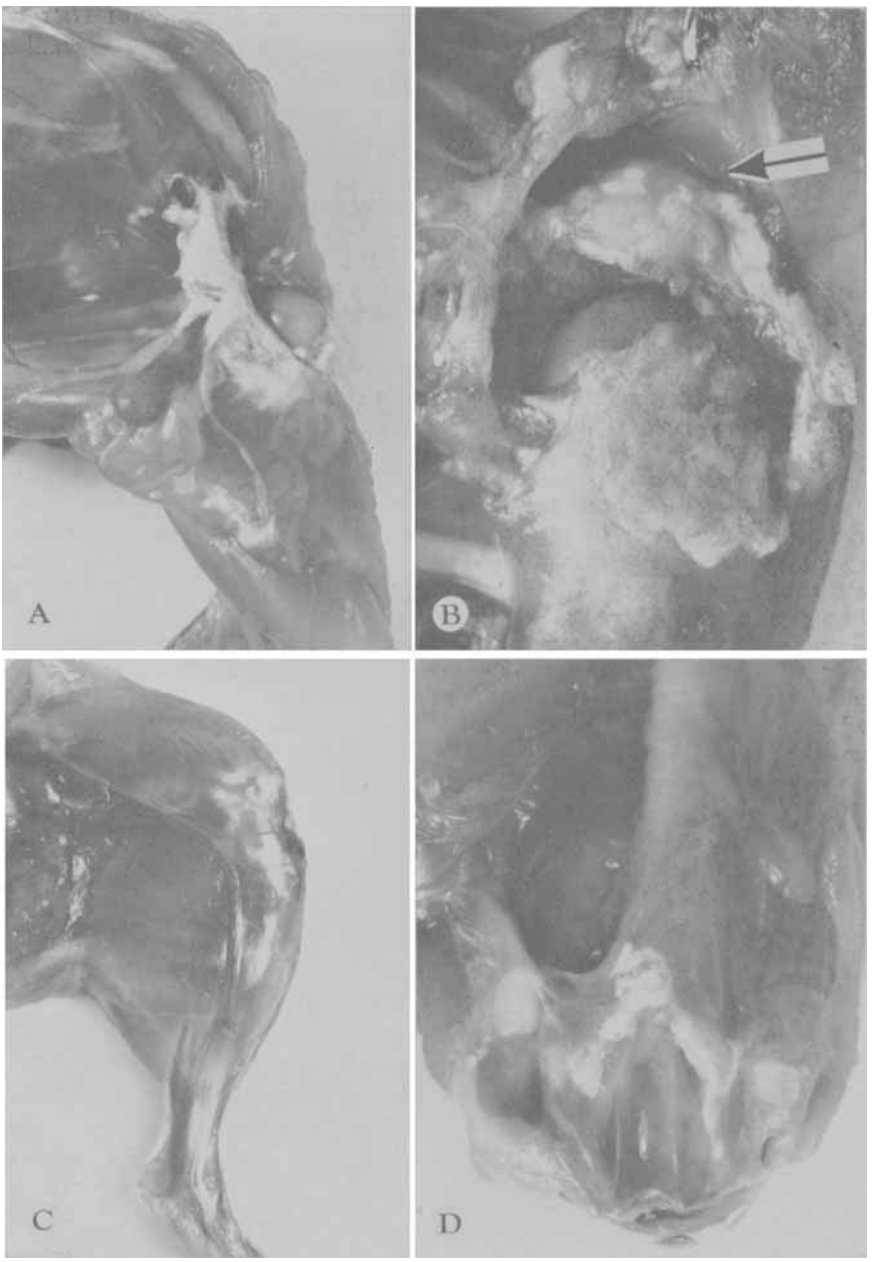

Fig. 1.—A. Ring-shaped calcium deposition in and around the shoulder joint. The head of the humerus is exposed to show its intact cartilage. **B.** Shoulder joint opened so as to expose subacromial bursa (arrow). White calcium deposits are clearly visible throughout the joint region. **C.** Calcium deposition in muscles and tendons of knee joint. **D.** Calcification in capsule of the knee joint along its attachment on femur.

every animal—whether it died spontaneously at an early stage or was killed at the termination of the experiment—showed extensive periarticular calcification in the subdeltoid region. Here, the calcium salts were deposited mainly

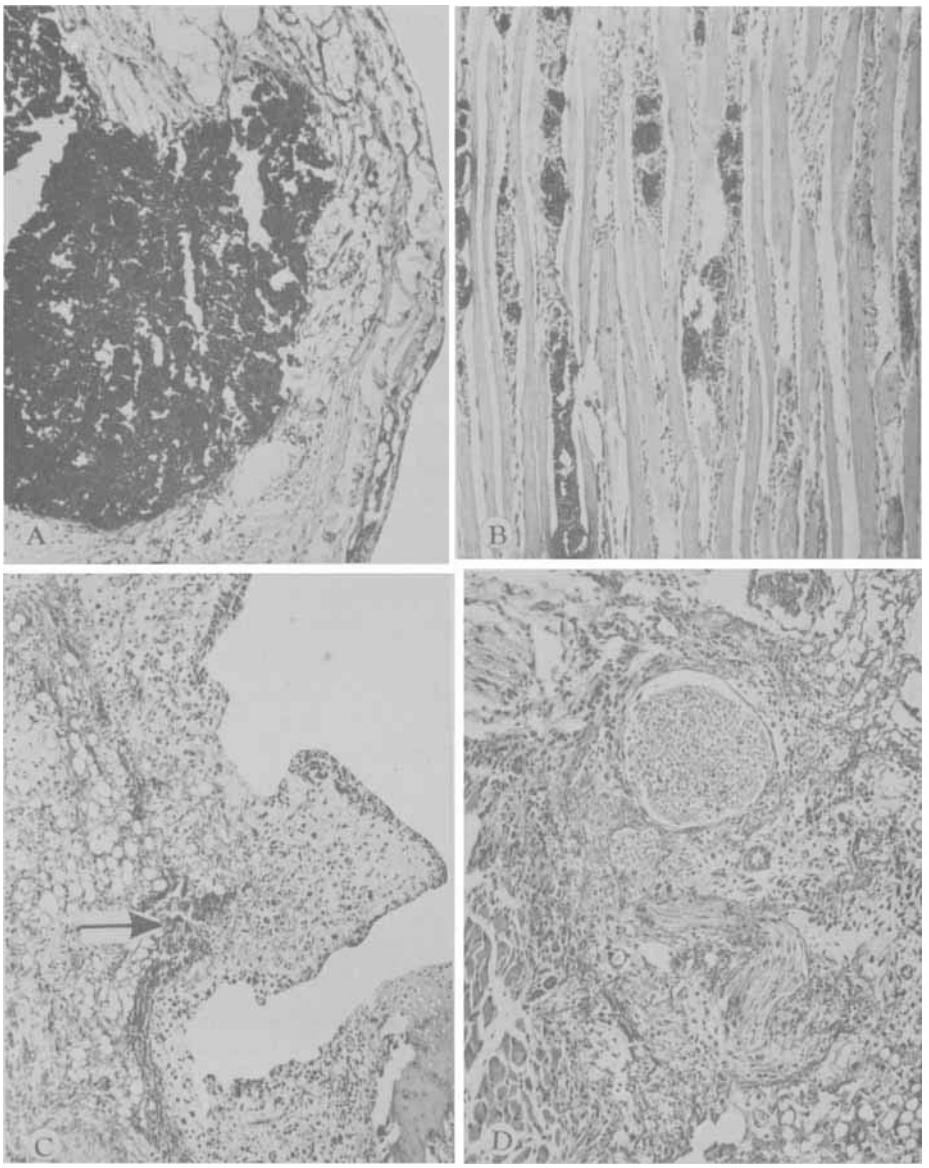

Fig. 2.—A. Calcified connective tissue focus in wall of subdeltoid bursa (v. Kossa X 120). **B.** Calcified muscle foci in deltoid (v.Kossa X 120). **C.** Pronounced inflammatory changes in synovial villi and somewhat basophilic necrotic band (arrow) which was presumably calcified, though calcium cannot be seen on this decalcified section (PAS X 120). **D.** Intense inflammatory infiltration around branches of brachial plexus in vicinity of calcified shoulder joint (PAS X 120).

in and round the subdeltoid bursa, but also in the adjacent tendons and muscles, which consequently assumed a chalky consistency and white color. Upon opening the bursa, a milky or gritty, white material was found in the lumen.

Histologically, all these white precipitates corresponded to v. Kóssa-positive calcium salts. In the same regions, there was inflammation and sclerosis, presumably as a secondary consequence of the calcium deposition which acted as

an irritating foreign body. Inflammatory changes were also seen in the synovial membranes and fat pads of the shoulder joint, but the joint cartilage itself was but rarely affected.

Calcification with inflammatory changes was also frequently observed in other joints, particularly those of the elbow, wrist, hip and knee. Similar lesions occurred somewhat less frequently round the sciatic nerves at the point of their emergence from the pelvis as well as between the greater trochanter of the femur and the tuberosity of the ischium. Sometimes the tendon insertions on this tuberosity were also calcified.

Discussion

There can be no doubt that, following sensitization by DHT, calciphylactic challenge with Thorotrast can produce a close simile of calcareous subdeltoid bursitis in the rat. It remains to be seen why here—unlike in the corresponding disease of man—the lesions in the shoulder were almost invariably accompanied by similar changes in other joints as well as by scleroderma-like plaques in the skin and esophagus. Of course, we have purposely used very large doses of calciphylactic agents in order to produce pronounced lesions regularly and in a short time, since our object was to develop an experimental simile of the disease that can be induced predictably, and in a short time. Despite the often widespread lesions that resulted, the shoulder affection was the most constant, often appearing even in comparatively resistant animals that showed little or no detectable changes elsewhere. It is conceivable, therefore, that, under the less drastic conditions prevailing in human pathology, an essentially related pathogenetic process could induce calcareous subdeltoid bursitis without other noteworthy morbid lesions.

The mechanisms of calciphylactic reactions in general, and of the form that leads to calcareous subdeltoid bursitis in particular, are still unknown. However, our earlier observations suggest that the diverse forms of calciphylaxis are due to a phenomenon of "vital mordanting" in which the sensitizing agent (here, DHT) induces a general tendency for calcium deposition, while the challenger (here, Thorotrast) prepares certain tissues for the local precipitation of calcium.[9,12] Histologic studies of the receptive tissues in rats given Thorotrast without previous DHT sensitization confirmed the presence of thorium dioxide crystals in the local histiocytes. It is conceivable that, in man, the deposition of endogenous or exogenous challengers (comparable in their biologic actions to Thorotrast) might act as a mordant for the precipitation of calcium, especially in individuals predisposed by a systemic calcifying tendency comparable to that experimentally induced by DHT.

In closing, it should be strongly emphasized that we have no proof of any relationship between the experimental syndrome and the spontaneous, calcareous subdeltoid bursitis of man; indeed, it would be futile even to enumerate arguments for or against this point since nothing is known about the etiology of the clinical condition. We have described this singular type of calciphylaxis only, in the hope that it may serve as a convenient model for the analysis of those factors that can produce a form of calcareous bursitis and as a test object for the screening of possible preventive or curative procedures.

SUMMARY

A calcareous subdeltoid bursitis can be regularly produced in the rat through calciphylactic challenge by Thorotrast after sensitization with dihydrotachysterol (DHT). Under the comparatively drastic conditions of these experiments, the shoulder lesions were usually accompanied by calcification elsewhere, especially in and around other joints, muscles, the skin and the esophagus, although subdeltoid bursitis was the most constant change.

This form of calciphylaxis is described merely as an experimental model of calcereous subdeltoid bursitis without implying that a calciphylactic mechanism is necessarily responsible for structurally comparable lesions in man.

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